Influence of isolate pathogenicity on the aerosol transmission of *Porcine reproductive and respiratory syndrome virus*

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Abstract

The objectives of this study were to evaluate the role of isolate pathogenicity in the aerosol transmission of *Porcine reproductive and respiratory syndrome virus* (PRRSV) and to determine whether PRRSV could be detected in air samples. To assess transmission, we exposed naïve recipient pigs to aerosols from pigs inoculated with PRRSV MN-30100, an isolate of low pathogenicity, or MN-184, a highly pathogenic isolate. Blood samples and nasal-swab samples were collected from the inoculated pigs during the exposure period and tested for the presence of PRRSV RNA by quantitative (real-time) reverse-transcriptase polymerase chain reaction (RT-PCR); the amount of RNA was expressed as the median tissue culture dose per milliliter ($TCID_{50}$/mL). The recipient pigs were clinically evaluated for 14 d after exposure and tested on days 7 and 14 by qualitative RT-PCR and enzyme-linked immunosorbent assay (ELISA). To prove the presence of PRRSV in aerosols, air samples were collected from each recipient-pig chamber by means of an air sampler. The PRRSV RNA concentrations were significantly higher ($P = 0.01$) in the blood samples from the pigs infected with PRRSV MN-184 than in the blood samples from those infected with PRRSV MN-30100; however, the concentrations in the nasal-swab samples were not significantly different ($P = 0.26$). Recipient pigs exposed to aerosols from pigs infected with PRRSV MN-184 became infected, whereas those exposed to aerosols from pigs infected with PRRSV MN-30100 did not; the difference in transmission rate was significant at $P = 0.04$. We detected PRRSV MN-184 RNA but not PRRSV MN-30100 RNA in air samples by PCR. Under the conditions of this study, PRRSV isolate pathogenicity may influence aerosol transmission of the virus.

Résumé

Les objectifs de la présente étude étaient d'évaluer le rôle de la pathogénicité de l'isolat dans la transmission par aérosol du virus du syndrome reproducteur et respiratoire porcin (PRRSV) et déterminer si le PRRSV pouvait être détecté dans des échantillons d'air. Pour évaluer la transmission, des porcs receveurs naïfs ont été exposés à des aérosols provenant de porcs infectés avec un isolat de PRRSV faiblement pathogène, MN-30100, ou un isolat hautement pathogène, MN-184. Des échantillons sanguins et des écouvillons nasaux ont été prélevés des porcs infectés durant la période d'exposition et testés pour la présence d'ARN du PRRSV par une réaction quantitative d'amplification en chaîne par la polymérase (temps réel) utilisant la transcriptase inverse (RT-PCR); la quantité d'ARN a été rapportée comme étant la dose médiane infectant une culture cellulaire par millilitre ($TCID_{50}$/mL). Les porcs receveurs ont été évalués cliniquement pendant 14 j suite à l'exposition et testés aux jours 7 et 14 par une épreuve RT-PCR qualitative et par épreuve immunoenzymatique (ELISA). Afin de confirmer la présence de PRRSV dans les aérosols, des échantillons d'air ont été prélevés de chaque chambre où étaient logés les porcs receveurs au moyen d'un échantillonneur d'air. Les concentrations d'ARN du PRRSV étaient significativement plus élevées (P = *0,01) dans les échantillons de sang provenant des porcs infectés avec PRRSV MN-184 que dans les échantillons de sang provenant des animaux infectés avec le PRRSV MN-30100; toutefois, les concentrations dans les écouvillons nasaux n'étaient pas significativement différentes* (P = *0,26). Les porcs receveurs exposés aux aérosols des porcs infectés avec le PRRSV MN-184 sont devenus infectés mais pas ceux exposés aux aérosols provenant des porcs infectés avec le PRRSV MN-30100; la différence dans le taux de transmission était significative* (P = *0,04). La présence d'ARN du PRRSV MN-184 dans l'air a été détectée par PCR mais pas celle du PRRSV MN-30100. Dans les conditions expérimentales de la présente étude, la pathogénicité de l'isolat de PRRSV pourrait être un facteur influençant la transmission par aérosol de ce virus.*

(Traduit par Docteur Serge Messier)

Introduction

Since its emergence, *Porcine reproductive and respiratory syndrome virus* (PRRSV) has remained a difficult and costly pathogen to control in the global swine industry (1). Despite effective eradication methods, reinfection of farms with new isolates has been problematic (2–4). Reported routes of indirect PRRSV transmission between farms include insects, transport vehicles, and aerosols (5–7). Transmission of PRRSV by aerosols has been reported to occur under experimental conditions over distances ranging from 0.5 to 150 m (8–10). Data from a large-scale epidemiologic study suggested that aerosol transmission is an important component of indirect PRRSV transmission throughout swine-producing regions (11).

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Despite the experimental evidence for aerosol transmission of PRRSV, attempts to document the spread of the virus through aerosols under controlled field conditions have not been successful (12–14). These studies involved large populations of experimentally infected pigs, commercial conditions, and a consistent viral isolate, PRRSV MN-30100, which was recovered from a persistently infected sow at a commercial farm (15). Following its use in several experiments, PRRSV MN-30100 was classified as being of low pathogenicity on the basis of its production of mild clinical signs after intranasal inoculation in growing pigs (12–18). Animals infected with PRRSV MN-30100 typically demonstrate a low-grade fever (temperature 40.2°C to 41.9°C), transient depression and anorexia (at 24 to 48 h after inoculation), and a minimal risk of death (15).

Being an RNA virus, PRRSV is able to undergo genetic change through mutation and recombination, and this has resulted in the generation of highly pathogenic isolates (19–24). Clinical signs exhibited after experimental intranasal inoculation with these isolates include high fever (temperature 42°C or greater), severe anorexia and depression, and a higher risk of lung lesions and death (22,23). Furthermore, replication is faster, resulting in higher concentrations of virus in blood and tissues (24).

Besides its clinical impact, PRRSV isolate pathogenicity may also influence the degree of virus transmission and aerosol shedding. In a previous study, variation in seroconversion rates, recovery of virus, and transmission to sentinels was observed between pigs infected with either a virulent field isolate or an isolate of lesser pathogenicity (25). Another study indicated that although PRRSV isolate pathogenicity did not significantly influence the concentration of the virus in aerosols, it did significantly affect the frequency of aerosol shedding (26).

In today's commercial production systems, it is not uncommon to house animals from different sources in a common farm site. The resultant comingling allows genetically and clinically diverse isolates of PRRSV to coexist and circulate throughout the population (27). Although it has been speculated that populations of this nature generate infectious aerosols that serve as sources of local spread of PRRSV between farms (11), the role of PRRSV isolate pathogenicity in the aerosol transmission of the virus remains unknown. To better understand the potential for PRRSV to be transmitted in aerosols, we assessed the impact of PRRSV isolate pathogenicity on the aerosol transmission of the virus and whether aerosolized PRRSV could be recovered with an air sampler. The study was based on the hypothesis that higher pathogenicity would significantly increase the rate of transmission of PRRSV in aerosols.

Materials and methods

Study site and animal source

The study was conducted at the University of Minnesota Swine Disease Eradication Center research farm, in west central Minnesota, USA, with 46 pigs 2 mo of age, weighing 25 kg, purchased from a farm designated PRRSV-negative on the basis of 10 y of diagnostic data and the absence of clinical signs. Upon arrival at the study site, the pigs were acclimatized for 3 d, and blood samples were collected and tested by qualitative polymerase chain reaction (PCR) to ensure the pigs' PRRSV-negative status (28). The pigs were housed in a total confinement facility that was mechanically ventilated and had partially slatted concrete floors. Throughout the study, the animals were cared for according to guidelines established by the University of Minnesota Institutional Animal Care and Use Committee.

Virus source

The viruses selected for the study were PRRSV MN-30100, an isolate of low pathogenicity, and PRRSV MN-184, an isolate of high pathogenicity. The PRRSV MN-184 had originally been obtained from an infected farm experiencing severe reproductive disease and an elevated sow mortality rate. This isolate had spread throughout pig farms in southern Minnesota during the winter of 2001. Clinical signs after intranasal inoculation included prolonged depression and anorexia and high fever (temperature greater than 42°C) (24). A recent evaluation in 25-kg pigs indicated that the animals infected with PRRSV MN-184 had significantly higher virus concentrations in blood and tonsil tissues than the animals infected with PRRSV MN-30100 (26).

Facilities

For this study, 2 experimental animal facilities were constructed and placed in separate buildings at the farm. Each facility consisted of 2 chambers, each 1.3 m in length and width and 1.8 m in height, that were connected via a 1.3-m-long rectangular duct 650 × 650 mm. The chambers and ducts were constructed with the use of 1.25-cm-thick sheets of recycled plastic (Snow-white board; Environmental Control Systems, Morris, Minnesota, USA) reinforced with a frame consisting of lengths of treated plywood 5 × 5 cm. The junctions between the frame and the plastic sheets were caulked with silicone. Chamber 1 had an air intake portal 0.4 m^2 with an airflow rate of 30 ft^3/min per 25-kg pig. Chamber 2 had a hole 20 cm in diameter to allow the air to be exhausted. Water was available to the pigs at all times. The maximum number of pigs allowable in each chamber was 5, for a stocking density of 0.29 m^2 per pig. While in the chamber, the pigs were able to easily move about, lie down, and turn around.

Experimental design

Upon confirmation of PRRSV-naïve status, the pigs were separated and housed in 1 of 4 groups: 10 to be inoculated with PRRSV MN-30100, 10 to be inoculated with PRRSV MN-184, 20 to serve as recipients, and 6 to serve as protocol controls to monitor for the unintentional mechanical transmission of PRRSV (5 to be inoculated with sterile saline and 1 to be housed for 6 h in the chamber facility before initiation of the trials). The recipient pigs were housed in different rooms, which prevented contact with each other and with the infected pigs.

The experiment was conducted twice, 5 pigs per isolate each time. Thus, 10 replicates were conducted for each isolate, 1 recipient pig representing 1 replicate. This sample size allowed for detection of a 30% infection rate at an alpha level of 0.05 and a study power of 80%.

On day 0, pigs were inoculated intranasally with 2 mL of PRRSV MN-30100 or PRRSV MN-184 at a median tissue culture dose per milliliter ($TCID_{50}$/mL) of 1×10^4 or with 2 mL of sterile saline. On days 3, 4, 5, 6, and 7 after inoculation, the 5 pigs inoculated with PRRSV MN-30100 were placed in chamber 1 of 1 facility, the 5 pigs inoculated with PRRSV MN-184 were placed in chamber 1 of the other facility, and 1 pig inoculated with sterile saline was placed

in chamber 2 of each facility. The 2 facilities were tested concurrently. The pigs were housed in the chambers for 6 h. During this exposure period, air was ventilated from chamber 1 to chamber 2 via the connecting duct. At the end of the 6 h, the inoculated pigs were returned to their pens, and the recipient pigs were placed in individual isolated facilities on the farm for assessment of PRRSV status after exposure. During the exposure period (days 3 to 7 after inoculation), the inoculated groups were rotated on alternate days between the facilities to minimize bias, and a different recipient pig was used each day. At the end of the 5-d exposure period, the inoculated pigs were euthanized and the recipients evaluated for 14 d. The experiment was then repeated with the 2nd groups of 5 PRRSV-infected pigs and the remainder of the recipients.

Biosecurity protocols

Strict biosecurity protocols were followed at all times to minimize the risk of contamination between groups. The chambers were sanitized between replicates with the use of procedures and products well documented to be effective in eliminating PRRSV from the environment (29). By means of a low-pressure foamer (Hydrofoamer; HydroStream, Cincinnati, Ohio, USA), 30 mL of a combination of 7% glutaraldehyde and 26% quaternary ammonium chloride (Synergize; Preserve International, Atlanta, Georgia, USA) added to 3840 mL of water was applied to all interior surfaces of the chambers. After drying, the walls, floor, and ceiling of all chambers were swabbed with sterile Dacron swabs (Fisher Scientific, Hanover Park, Illinois, USA). The swabs were drawn over each surface in a left-to-right zigzag pattern, placed in sterile plastic tubes (Falcon, Becton-Dickinson, Franklin Park, New Jersey, USA), each containing 1 mL of sterile cell culture medium (minimum essential medium [MEM]; Difco, Detroit, Michigan, USA), and tested for the presence of PRRSV RNA by qualitative TaqMan PCR (Perkin-Elmer Applied Biosystems, Foster City, California, USA). Recipient pigs were handled before inoculated pigs, and personnel washed their hands and changed gloves, boots, and coveralls between pigs (30,31). Separate traffic patterns for the transfer of inoculated and recipient pigs were established to prevent contact between personnel. The trials were conducted during the winter to eliminate the risk of insect transmission between pens and rooms. Footwear was sanitized by a 10-s immersion in foot baths of 6.5% sodium hypochlorite (Clorox; Clorox Company, Oakland, California, USA) located outside the doorway of each room (31).

Diagnostic monitoring

Blood and nasal swabs were collected from all the inoculated pigs to monitor experimental infection and shedding. By collecting from a different pig each day, we ensured that all the experimentally infected pigs in each virus group were sampled once during the 5-d exposure period. Nasal samples were collected by inserting a sterile swab 2 cm into each of the nares. The swab was placed in a sterile plastic tube containing 2 mL of sterile saline. The concentration of PRRSV RNA in all samples was determined by TaqMan quantitative (real-time) reverse-transcriptase PCR (RT-PCR) at the Minnesota Veterinary Diagnostic Laboratory, by a modification of the protocol previously described (32). Data from serum and swabs were expressed as $TCID_{50}$/mL. A standard curve was developed by making 10-fold dilutions (1×10^{-6} to $1 \times 10^{4.6}$ $TCID_{50}$/mL) of PRRSV MN-30100 and PRRSV MN-184. Each sample was run in triplicate, and the mean RNA concentration for serum and swabs for each isolate was calculated from these values. Blood was collected from the recipient pigs 7 and 14 d after exposure and tested by qualitative PCR and enzyme-linked immunosorbent assay (ELISA), the latter being used just for the day-14 samples. The protocol-control pigs were blood-tested by PCR and ELISA at the end of the exposure period. Swabs from the sanitized chambers were tested for the presence of PRRSV RNA by qualitative PCR.

Statistical analysis

The effect of isolate pathogenicity on the PRRSV RNA concentrations in blood and nasal-swab samples was evaluated by means of a generalized analysis of variance. Differences in the proportions of recipient pigs infected, according to PRRSV isolate, were assessed for significance by the 2-tailed Fisher's exact test.

Air sampling

We had planned that if aerosol transmission was established during the 1st phase of the study, we would conduct a 2nd phase to verify the presence of PRRSV in air collected from the recipient chamber. For this additional phase, 5 PRRSV-naïve pigs were inoculated with PRRSV MN-30100 and 5 with PRRSV MN-184 by the same procedures as used in the 1st phase. As before, on days 3, 4, 5, 6, and 7 after inoculation, the pigs were housed in chamber 1 for 6 h, the facilities being in separate buildings on the farm. Samples from the chamber-2 airspace were collected with a high-volume air sampler (Midwest Micro-Tek, Brookings, South Dakota, USA) placed in the chamber on a stool approximately 4 m off the ground and 1 m from the junction of the duct and chamber 2 in order to contact the air exhausted from the duct and dispersed into chamber 2. The sampler, based on the principles of an air centrifuge, collected 800 L of air per 2-min sampling period, during which 10 mL of MEM was added to the rotating drum, mixing with particles in the collected air. On days 3 to 7 after inoculation, 10 air samples were collected at 60, 180, and 360 min after the onset of each 6-h replicate. The 3 samples were pooled by collection day, and a 5-mL aliquot was removed and frozen at –20°C. All aliquots (5 per isolate) were tested by TaqMan qualitative PCR for the presence of PRRSV RNA. Nucleic acid sequencing was performed on all positive samples to verify which isolate was present in the sample.

Results

All pigs were determined to be PRRSV-naïve upon arrival at the farm. In the recipient chambers of both facilities, the mean airflow velocity was 5.0 m/s, temperature 20°C, and relative humidity 48%. Successful experimental infection and shedding of the 2 isolates were documented: all pigs were viremic and had detectable levels of PRRSV RNA in the collected samples. The mean concentration of PRRSV RNA in the nasal-swab samples was 1.2×10^0 (range 3×10^{-1} to 5.3×10^1) $TCID_{50}$/mL for the pigs inoculated with PRRSV MN-30100 and 1.0×10^1 (range 3.3×10^0 to 2.2×10^1) $TCID_{50}$/mL for the pigs inoculated with PRRSV MN-184. The mean concentrations of PRRSV RNA in the blood samples were 6.1×10^1 (range 4.5×10^0 to 1.2×10^3) and 3.4×10^2 (range 1.7×10^0 to 1.5×10^3)

$TCID_{50}$/mL, respectively. The PRRSV RNA levels in the blood samples were significantly higher in the pigs infected with PRRSV MN-184 ($P = 0.01$) than in those inoculated with PRRSV MN-30100; there was no significant difference between the RNA levels in the nasal-swab samples ($P = 0.26$).

Clinical signs in the pigs inoculated with PRRSV MN-30100 included transient elevation of temperature to 39°C to 40°C, anorexia at 24 to 28 h after inoculation, and mild depression. Clinical signs in the pigs inoculated with PRRSV MN-184 were more severe and of longer duration than those in the pigs inoculated with PRRSV MN-30100 and included elevation of rectal temperature above 42°C, along with severe anorexia and depression. None of the animals inoculated with either isolate died.

Transmission of PRRSV by aerosol was detected in 4 of the 10 replicates involving pigs inoculated with PRRSV MN-184. Similar clinical signs were observed in the infected recipients. Transmission was observed on days 5 and 7 after inoculation of the pigs in group 1 and on days 6 and 7 after inoculation of the pigs in group 2. The 4 recipients were confirmed to be infected by the day-7 PCR results and the day-14 PCR and ELISA results. In contrast, no evidence of aerosol transmission of PRRSV was observed in the 10 replicates involving pigs inoculated with PRRSV MN-30100: the recipient pigs remained negative by PCR and ELISA during the monitoring period. This difference in the rate of transmission was significant at $P = 0.04$. All the protocol-control pigs remained negative throughout the study, and all swabs from the interior of the chambers were PCR-negative.

Of the 5 pooled samples of air collected on days 3 to 7 after inoculation with PRRSV MN-184, only those originating on days 4, 5, and 7 yielded PRRSV RNA. The presence of this isolate was verified by nucleic acid sequencing of the open reading frame 5 region (33). Positive results were not obtained for samples of air originating from pigs inoculated with PRRSV MN-30100.

Discussion

These results suggest that isolate pathogenicity may be a factor in the aerosol transmission of PRRSV. Recipient pigs became infected with PRRSV MN-184 late in the exposure period, possibly because the experimentally infected pigs were shedding larger quantities of virus later in the postinoculation period. The presence of PRRSV MN-184 in samples of air originating from the experimentally infected pigs and collected from chamber 2 over the same period supports this conclusion, although demonstration of transmission was not an objective of the additional study.

Since evidence of potential aerosol transmission was observed in the 1st phase, it became imperative to demonstrate the presence of strain-specific PRRSV in air originating from experimentally infected pigs to further support the fact that the virus traveled from chamber to chamber via air. It was also important to rule out the possibility that infection of recipient pigs was due to contamination of the facilities. The chambers were sanitized with the use of procedures and products well documented to be effective in eliminating PRRSV from the environment (29), and swabs collected from the interior surfaces of all chambers were PCR-negative. Furthermore, none of the protocol-control pigs became infected during the study. Surprisingly, the mean concentration of PRRSV RNA in nasal-swab samples did not differ significantly by isolate, in contrast to the significant differences in the blood samples. However, the inability to collect nasal-swab samples daily from the experimentally infected pigs, owing to animal-welfare issues and budget constraints, may have influenced this outcome.

A limitation of the study was that only 2 PRRSV isolates were tested. Therefore, despite the fact that PRRSV MN-184 and PRRSV MN-30100 differed in both pathogenicity and replication rate and seemed to be logical candidates for this initial evaluation (15–18,24,26), our results should not be generalized across other isolates without further investigation. Differences in transmission and detection of PRRSV in air could also have been due to timing issues within the experimental design, such as the 5-d exposure period and the 6-h period that the recipients spent in the chambers. Also, only 10 replicates were conducted per isolate, which allowed for only a 30% detection rate. Had we used more replicates or extended the sampling period beyond 7 d, aerosol transmission of PRRSV MN-30100 might have occurred.

Owing to their superior sensitivity compared with virus-isolation techniques, PCR assays were used to both qualitatively and quantitatively assess the presence of PRRSV RNA. However, the results were not representative of the infectious dose or the presence of infectious virus.

Finally, since only 3 of the 5 air samples originating from pigs inoculated with PRRSV MN-184 and 0 of 5 air samples originating from pigs inoculated with PRRSV MN-30100 were positive for PRRSV RNA, it is possible that the sensitivity of the air sampler influenced virus detection or that sampling was not conducted during periods of active shedding. However, since transmission was evident only in the group inoculated with PRRSV MN-184, the importance of this potential limitation is minimal.

In closing, although these data provide insight into the potential role of isolate pathogenicity in the transmission of PRRSV by aerosol, research at the population level, including large-scale epidemiologic studies, is necessary to better understand the importance of this factor, along with other potentially important risk factors (population size, area density of swine farms, and environmental parameters), in the aerosol transmission of PRRSV in the field. Once this information is available, swine producers and practitioners will be able to assess the risk of this route of transmission and thereby design cost-effective biosecurity strategies to minimize the risk of PRRSV spread between farms by aerosol and enhance the success of regional PRRS control and eradication projects.

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